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Removal of toxic metals from solution by leaf, stem and root phytomass of *Quercus ilex* L. (holly oak)

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"Capsule": *Holly oak phytomass was useful as a biofilter of toxic trace metals.*

Abstract

Increased consciousness for safeguarding the aqueous environment has prompted a search for alternative technologies for the removal of toxic metal ions from aqueous solutions. In this regard, a wide variety of biomass is being considered as adsorbents of heavy metals for treatment of industrial and domestic wastewaters as well as natural waters, including drinking water. In the present investigation, the potential of *Quercus ilex* phytomass from stem, leaf and root as an adsorbent of chromium (Cr), nickel (Ni), copper (Cu), cadmium (Cd) and lead (Pb) at ambient temperature was investigated. The metal uptake capacity of the root for different metals was found to be in the order: Ni > Cd > Pb > Cu > Cr; stem Ni > Pb > Cu > Cd > Cr; and leaf Ni > Cd > Cu > Pb > Cr. The highest amount adsorbed was Ni (root > leaf > stem). Data from this laboratory demonstrated that Ni is sequestered mostly in the roots, where concentrations can be as high as 428.4 ng/g dry wt., when 1-year-old seedlings were treated with Ni (2000 mg/l) in pot culture experiments, compared to 7.63 ng/g dry wt., control (garden and greenhouse soil) topsoil where Ni was present in trace amounts. This proves that the root biomass of *Q. ilex* has the capacity for complexing Ni. Cr exhibited the least adsorption values for all the three types of phytomass compared to other metals. The trend of adsorption of the phytomass was similar for Ni and Cd, i.e. root > leaf > stem. Desorption with 10 mM Na_4 EDTA was effective (55–90%) and, hence, there exists the possibility of recycling the phytomass. The biosorption results of recycled phytomass suggest that the selected adsorbents are re-usable. The advantages and potential of the *Q. ilex* phytomass as a biofilter of toxic trace metals, the scope and need for enhancing the efficiency of the *Q. ilex* phytomass as an adsorbent of metals are presented.

Keywords: *Quercus ilex*; Chromium; Nickel; Copper; Cadmium; Lead; Stem; Root; Phytomass; Biosorption; Desorption

1. Introduction

Heavy metals are released into the aqueous environment through a variety of sources such as metal smelters, effluents from plastics, textiles, microelectronics, wood preservatives-producing industries, usage of fertilizers and pesticides (Ross, 1994; Prasad and Hagemeyer, 1999). Natural waters also contain toxic metals depending upon the bed rock (Greger 1999).

The cost of decontamination of toxic metals from industrial effluents and wastewaters using ion exchange resins is exorbitant. Hence, that the usage of indigenous biodegradable resources for treating hazardous waste would be less expensive is relevant to regional emerging environmental biotechnology. The concentration limits for classification of hazardous waste-containing metals vary for different metals (Table 1). To clean up metals in contaminated waters, a wide variety of biomass, including bacteria, fungi (free and immobilized), algae, and higher plants (agricultural and forestry wastes), have been tested as adsorbents (Table 2).

Quercus ilex L. (holly oak) is an evergreen tree which plays an important role in western Mediterranean ecosystems. It is the only tree growing in the serpentine area of about 8000 ha in northeast Portugal. The serpentine soils are very poor in essential nutrients and high in toxic metals such as chromium (Cr), cobalt (Co), nickel (Ni) (Sequeira and Pinto da Silva, 1991). In the urban area of Naples in Italy, *Q. ilex* has been used for trace metal biomonitoring in the soil environment (Alfani et al., 1996). Hence, *Q. ilex* phytomass (stem,

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Table 1
Concentration limit (international standards) of some metals for classification as hazardous waste (Balköse and Baltacioğlu, 1992)

Metal	Water (mg/l) or soil (mg/kg)
Cu	2.5
Zn	20
Ni	20
Cd	1
Hg	0.2

leaf, root), which is widely available in southern Europe, was tested for its ability to adsorb selected metals, such as cadmium (Cd), Cr, copper (Cu), Ni, and lead (Pb) from aqueous solutions.

2. Materials and methods

2.1. Phytomass

Q. ilex leaf, stem and root samples (3-year-old plants) were collected from the experimental plots located in the botanical garden, Department of Botany, University of Coimbra (Jardim Botânico, Departamento de Botânica, Universidade de Coimbra, Coimbra, Portugal). Samples were oven dried at 55°C overnight. Dried material was ground to homogenous powder using mortar and pestle with the help of liquid nitrogen

2.2. Biosorption

The homogenously powdered phytomass 1 g (particle size smaller than 200 μm) was added to 100 ml containing 10 mg/l metal solution. The metal solutions (Cr, Ni, Cu, Cd, Pb) were prepared by dilution of 1000 mg/l atomic absorption standard solutions procured from Reagecon, Ireland. The samples were stirred in separate beakers at room temperature (22 ± 2°C) for 1 h. Addition of phytomass to metal ion-containing solution resulted in different initial pH (pH_i) after 1 h. The pH was adjusted to facilitate adsorption with 0.1 N NaOH or 0.1 N H_2SO_4. During the experimentation of biosorption, the pH of the solutions was regularly monitored with a pH meter (Metrohm, 716 DMS Titrino, Switzerland). The experiment was continued for 3 days, and the final pH (pH_f) was recorded.

2.3. Sedimentation

Solutions containing metal ions and phytomass were stirred at regular intervals and allowed to settle. No cationic flocculants were used to facilitate sedimentation. The settled phytomass in metal ion-containing solutions could be observed by the naked eye. The clear supernatant was decanted into glass vials for analysis.

2.4. Desorption and recycling

After 3 days of biosorption, the clear supernatant was used for metal ion analysis and the phytomass was washed (×3) with deionized water and oven dried overnight. The dried phytomass was resuspended in 100 ml of 10 mM Na_4 EDTA for 6 h. The filtrate was analysed for determining the metal desorbed. Deionized water was used to wash the residue. The phytomass after one cycle was oven dried overnight. The same procedure stated previously for biosorption was followed to test the phytomass potential for re-use

2.5. Metal analysis

After 3 days of biosorption, the residual concentration of the metal ions was estimated by an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100). The experiment was repeated twice and results averaged. All glassware used was soaked in 10% nitric acid overnight and rinsed (×3) with deionized water before allowing to dry. The results of heavy metals adsorbed to stem, leaf, and root phytomass were quantified by mass balance following Vázquez et al. (1994):

$$R = \frac{C_i - C_r}{C_i} \times 100$$

$$\frac{x}{m} = \frac{C_i - C_r}{m},$$

where R is the percentage of metal adsorbed from initial concentration (i.e. 10 mg/l in all the experiments), x/m is the mass of metal adsorbed/mass of the phytomass (stem, leaf, root) (mg/g), m is the concentration of phytomass in the solution (g/l), C_i is the initial concentration of metal at the start of the biosorption (mg/l) and C_r is the residual concentration of the metal (mg/l) after 3 days of biosorption.

3. Results

Various samples of phytomass in contact with metal-containing solution exhibited a wide range of pH values (Table 3) possibly due to the nature of the anions. To maximize the adsorption of the metals to phytomass, pH adjustment was performed. Initially, adsorption experiments conducted with Ni indicated that the pH and the duration of contact time of phytomass with aqueous solution were the important factors for the removal of metal having an initial concentration 10 mg/l. A value of pH 6 gave optimum results over a period of 6 h (Fig. 1). The highest adsorption values were obtained after 3 days. Further, prolongation of contact time to 6 days did not increase adsorption (Fig. 2). On

Table 2
Heavy metal removal and recovery from aqueous solutions using various kinds of biomass as adsorbent

Metal(s)	Adsorbent	Reference
Variety of heavy metals	Bark	Randall (1977); Randall et al. (1974, 1976)
	Agrowaste	Kumar and Dara (1982)
	Apple waste	Maranon and Sastre (1991)
	Cellulose	Shukla and Sakhardande (1991)
	Green algae Rice hulls	Roy et al. (1993)
	Immobilized *Aspergillus niger, A. oryzae*	Kapoor and Viraraghavan (1998)
Ni, Cu, Zn, Cd, Hg, Pb	Wool fibers	Balköse and Baltacioğlu (1992)
Cd, Cu, Pb, Ni	*Aspergillus niger*	Kapoor and Viraraghavan (1998)
Cr, Ni, Pb, Cd, Zn	Olive, *Olea europea*, mill waste	Gharaibeh et al. (1988)
Zn, Cu, Ni	*Streptomyces rimosus* (bacteria), *Saccharomyces cerevisiae* (yeast), *Penicillium chrysogenum* (fungi), *Fucus vesiculosus* and *Ascophyllum nodosum* (marine algae)	Bakkaloglu et al. (1998)
Pb, Ni, Cr, Cd, Cu	*Phanarochaete chrysosporium*, *P. versicolor*	Yetis et al. (1998)
U	*Pinus radiata*	Freer et al. (1989)
Hg	Cotton	Roberts and Rowland (1973)
	Coffee grounds	Macchi et al. (1986)
Cu	Groundnut skins	Randall et al. (1975)
	Immobilized *Pseudomonas putida* 5-X, *Aspergillus niger*	Chua et al. (1998) Townsley et al. (1986) Mullen et al. (1992)
	Mucor rouxxi	Mullen et al. (1992)
Zn, Cu, Pb	*Pinus pinaster* bark	Vázquez et al. (1994)
Cd	Actinomycetes, *Aspergillus niger A.oryzae*	Kefala et al. (1999) Mullen et al. (1992) Kiff and Little (1986) Huang et al. (1988)
	Rhizopus arrhizus, R. nigricans	Holan and Volesky (1995)
Pb, Ni	Marine algae	Holan and Volesky (1994)
Cr(VI)	*Rhizopus arrhizus*	Prakasham et al. (1999)
Pb	*Rhizopus arrhizus, R. nigricans, Phanarochaete chrysogenum*	Holan and Volesky (1995)
Ni	*Aspergillus niger, Rhizopus arrhizus*	Holan and Volesky (1995)

the third day, the adsorption capacity of the different samples of phytomass was determined (Table 4). The metal adsorption capacity of the root for different metals was found to be in the order: Ni > Cd > Pb > Cu > Cr; stem Ni > Pb > Cu > Cd > Cr; and leaf Ni > Cd > Cu > Pb > Cr. The highest amount adsorbed was Ni (root > leaf > stem). After determining the adsorption values, the solution was filtered and the phytomass

Table 3
The pH values of various types of phytomass with different metal-containing aqueous solutions

Metal	pH_i (after 1 h)			pH_f (after 3 days)		
	Stem	Leaf	Root	Stem	Leaf	Root
Pb	2.67	2.67	2.64	5.2	6.1	6.0
Ni	5.26	5.06	5.3	6.1	6.3	6.2
Cd	2.67	2.63	2.58	7.0	6.8	6.5
Cu	2.63	2.66	2.65	5.4	5.3	5.4
Cr	2.71	2.62	2.6	5.0	4.8	5.4

was oven dried and suspended in 100 ml of 10 mM Na_4 EDTA for 6 h. The amount of metal desorbed by the phytomass is shown in Table 5. The results indicate that the phytomass is re-usable. The deficit in recovery could be partly due to the loss of the phytomass (via digestion) and variations in solution physico-chemistry.

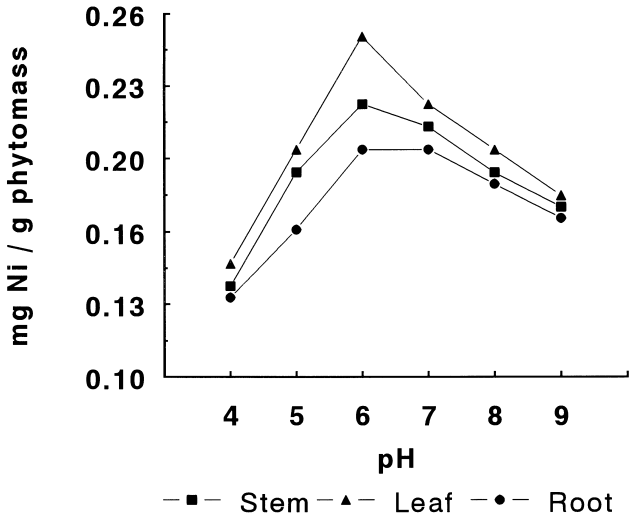

Fig. 1. Influence of pH on the removal of nickel (Ni) by the stem, leaf, and root phytomass of *Quercus ilex*.

4. Discussion

The principal metal-binding ligands reported in plant tissues encompass amino acids, oligo- and polypeptides (glutathione, phytochelatins, metallothioneins); macrocyclic agents (porphyrins, cobalamines, chlorophylls); polysaccharides and glycosides (rhamnogalacturonan); nucleobases, oligo-polynucleosides and nucleotides (DNA fragments) (Łobinski and Potin-Gautier, 1998). Several of these metal-binding bioligands were reported in the vascular tissues (xylem, phloem) of the plants. Most of the transitional and some main group metal ions form complexes with these bioligands (Prasad, 1998, 1999; Pohlmeier, 1999).

In an earlier investigation from this laboratory, Nabais et al. (1996) determined the Ni concentrations in the main stem of *Q. ilex* growing on serpentine and sandy loam soils. Nabais et al. (1997) quantified the amino acids and carboxylic acids in the xylem sap of *Q. ilex* and discussed their role in Ni binding and

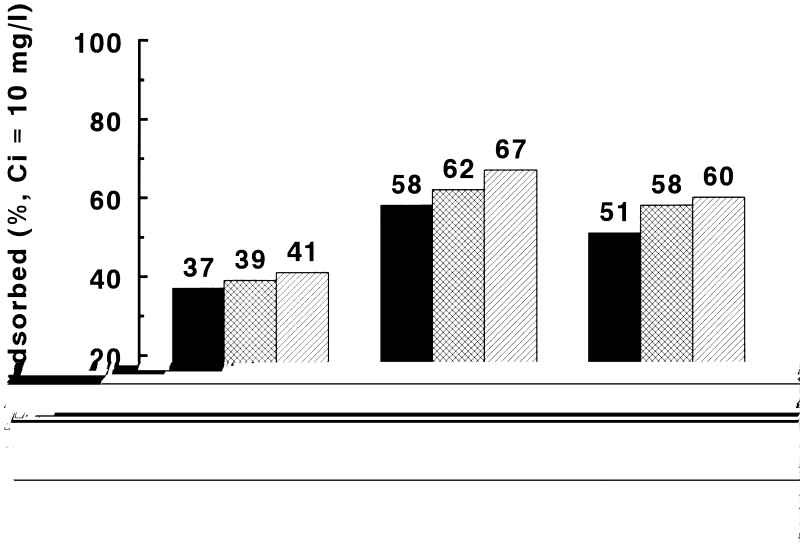

Fig. 2. Influence of contact time on the removal of nickel (Ni) by the stem, leaf, and root phytomass of *Quercus ilex*.

Table 4
Comparison of biosorption of metal ions on various types of phytomass of *Quercus ilex* phytomass

	Metal ion concentration		Biosorption efficiency (%)	Metal adsorbed	
	Initial (mg/l)	Final (mg/l)		mg/g	μmol/g
Pb					
Stem	10	2.52	74.8	0.75	3.6
Leaf	10	6.05	39.5	0.4	1.9
Root	10	4.4	56.0	0.56	2.7
Ni					
Stem	10	4.2	58.0	0.58	9.9
Leaf	10	3.83	62.0	0.62	10.6
Root	10	0.62	67.2	0.67	11.5
Cd					
Stem	10	6.38	36.2	0.36	3.2
Leaf	10	4.68	53.2	0.53	4.73
Root	10	4.0	60.0	0.6	5.3
Cu					
Stem	10	7.88	21.2	0.21	3.3
Leaf	10	8.0	20.0	0.20	3.14
Root	10	8.81	11.9	0.12	1.87
Cr					
Stem	10	9.32	6.8	0.07	1.3
Leaf	10	9.18	8.2	0.08	1.6
Root	10	9.08	9.2	0.09	1.77

Table 5
Desorption values and biosorption efficiency of recycled phytomass

Metal	Percentage desorbed with 10 mM Na_4 EDTA			Biosorption efficiency (%) of recycled phytomass		
	Stem	Leaf	Root	Stem	Leaf	Root
Pb	78	65	69	65	27	35
Ni	70	73	79	47	54	40
Cd	63	55	67	28	40	35
Cu	85	78	77	17	12	5
Cr	82	85	90	2	6	8

transport. The concentration of Ni in the xylem was as high as 5282.4 ng/g dry wt. in *Q. ilex* growing on serpentine soils as compared to 704 ng/g dry wt. in plants growing on sandy loam soils. This confirms that the phytomass of *Q. ilex* comprises Ni-binding and- transporting bioligands and would be of significance in phytoremediation of metal-contaminated soils and water (Prasad and Freitas, 1999).

Nabais and Freitas (Department of Botany, University of Coimbra, Coimbra, unpublished data) also developed a functional model for Ni transport and complexation in *Q. ilex* trees based on field and experimental data. Branches of *Q. ilex* growing on serpentine and sandy loam soils of northeast Portugal were collected every month for 1 year and the Ni concentrations in the xylem sap, leaves, xylem and phloem tissues were analyzed.

The average concentrations were:

	Serpentine soils (ng/g dry wt.)	Sandy loam (ng/g dry wt.)
Xylem	23.5	16
Phloem	24.5	18.3
Leaves	17.8	16

Nabais and Freitas (Department of Botany, University of Coimbra, Coimbra, unpublished data) also performed another experiment using 1-year-old *Q. ilex* trees growing in soil artificially contaminated with 500, 1000, or 2000 mg/l of total Ni. This experiment substantiated that Ni is mostly sequestered in the roots where concentrations can be as high as 428.4 ng/g dry wt., in the 2000 mg/l treatment, compared to 7.6 ng/g

dry wt., in the control. Xylem transport of Ni to the leaves was low. Leaves from the 2000 mg/l treatment showed an average concentration of Ni of 32.8 ng/g dry wt., compared to 11.2 ng/g dry wt. in the control, supporting Ni accumulation in leaves several times that in the control trees (Freitas, 1999).

Q. ilex phytomass is abundantly available in northeast Portugal. Different parts of the plant have different adsorption abilities due to localization of specific ligands which have an affinity to bind a particular metal (Table 4). For enhancing the ability of metal adsorption, chemical pretreatment might be helpful and would expose the metal-binding ligands in the chosen tissues (Vázquez et al., 1994). Physico-chemical parameters, such as temperature, pH of the contact solution and conditions of the reaction, e.g. continuous or batch mode, are reported to influence the adsorption results (Balköse and Baltacioğlu, 1992; Vázquez et al., 1994). The distribution coefficient of Ni, Cu, Zn, Cd, Hg and Pb was dependent on temperature when wool fibers were used as adsorbent. At 50°C Cu, Zn and Pb had higher values, whereas for Ni, Cd, and mercury (Hg) the distribution coefficient values were higher at 25°C (Balköse and Baltacioğlu, 1992). Hence, standardization of physico-chemical parameters and experimental conditions might be useful to optimize the efficacy of the chosen adsorbent.

5. Conclusions

This work, for the first time, emphasizes the potential of the *Q. ilex* phytomass as an adsorbent of toxic metal ions from solution. The *Q. ilex* phytomass is abundantly available and, hence, methodology based on its usage as adsorbent would be inexpensive, unlike some fungal-, algal- and microbial biomass-based reactors which require expensive culture media, and costly biosafety precautions. Further, the biosorption potential of *Q. ilex* root phytomass for Ni is quite promising. Utilization of *Q. ilex* phytomass and its relevance to metal chelation has tremendous scope for developing and designing innovative clean-up technologies. Accordingly, supplementary experimentation is being planned with *Q. ilex* aiming at the removal and recovery of toxic metal ions not only in natural and industrial wastewaters but also in metal-contaminated soils.

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References

Alfani, A., Bartoli, G., Rutigliano, F.A., Maisto, G., De Santo, A.V., 1996. Trace metal biomonitoring in the soil and the leaves of *Quercus ilex* in the urban area of Naples. Biological Trace Element Research 51, 117–131.

Bakkaloglu, I., Butter, T.J., Evison, L.M., Holland, F.S., Hancock, I.C., 1998. Screening of various types of biomass for removal and recovery of heavy metals (Zn, Cu, Ni) by biosorption, sedimentation and desorption. Water Science and Technology 38, 269–277.

Balköse, D., Baltacioğlu, H., 1992. A desorption of heavy metal cations from aqueous solutions by wool fibers. Journal of Chemical Technology and Biotechnology 54, 393–397.

Chua, H., Wong, P.K., Yu, P.H.F., Li, X.Z., 1998. The removal and recovery of copper ions(II) from wastewater by magnetite immobilized cells of *Pseudomonas putida* 5-X. Water Science and Technology 38, 315–322.

Freer, J., Baeza, J., Maturana, H., Palma, G., 1989. Removal and recovery of uranium by modified *Pinus radiata* D.Don bark. Journal of Chemical Technology and Biotechnology 46, 41–48.

Freitas, H., 1999. Binding of metal ions in the xylem of trees (*Quercus ilex*). Abstract No. 11.10.3. 16th Int. Bot. Congr. St. Louis, MO, USA. 1–7 August.

Gharaibeh, S.H., Abu-El-Shar, W.Y., Al-Kofahi, M.M., 1998. Removal of selected heavy metals from aqueous solutions using processed solid residue of olive mill products. Water Research 32, 498–502.

Greger, M., 1999. Metal availability and bioconcentration in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: From Molecules to Ecosystems. Springer, Berlin, pp. 1–28.

Holan, Z.R., Volesky, B., 1994. Biosorption of lead and nickel by biomass of marine algae. Biotechnology and Bioengineering 43, 1001–1009.

Holan, Z.R., Volesky, B., 1995. Accumulation of cadmium, lead, nickel by fungal and wood biosorbents. Applied Biochemistry and Biotechnology 53, 133–146.

Huang, C.P., Westman, D., Quirk, K., Huang, C.P., Morehart, A.L., 1988. Removal of cadmium (II) from dilute solutions by fungal biomass. Science and Technology 6, 405–419.

Kapoor, A., Viraraghavan, T., 1998. Removal of heavy metals from aqueous solutions using immobilized fungal biomass in continuous mode. Water Research 32, 1968–1977.

Kefala, M.I., Zouboulis, A.I., Matis, K.A., 1999. Biosorption of cadmium ions by Actinomycetes and separation by flotation. Environmental Pollution 104, 283–293.

Kiff, R., Little, D.R., 1986. Biosorption of heavy metals by immobilized fungal biomass. In: Eccles, H., Hunt, S. (Eds.), Immobilization of Ions by Biosorption. Ellis Horwood, Chichester, pp. 71–80.

Kumar, P., Dara, S.S., 1982. Utilization of agricultural wastes for decontaminating industrial/domestic wastewaters from toxic metals. Agriculture Wastes 4, 213–223.

Łobinski, R., Potin-Gautier, M., 1998. Metals and biomolecules — bioinorganic analytical chemistry. Analusis 26, 21–24.

Macchi, G., Marani, D., Tirivanthi, G., 1986. Uptake of mercury by exhausted coffee grounds. Environmental Technology Letters 7, 431–444.

Maranon, E., Sastre, H., 1991. Heavy metal removal in packed beds using apple waste. Bioresource Technology 38, 39–43.

Mullen, M.D., Wolf, D.C., Beveridge, T.J., Bailey, G.W., 1992. Sorption of heavy metals by soil fungi *Aspergillus niger* and *Mucor rouxxi*. Soil Biology and Biochemistry 24, 129–135.

Nabais, C., Freitas, H., Hagemeyer, J., Breckle, S.W., 1996. Radial distribution of Ni in stem wood of *Quercus ilex* L. trees growing on serpentine and sandy loam (umbric leptosol) soils of NE-Portugal. Plant Soil 183, 181–185.

Nabais, C., Freitas, H., Hagemeyer, J. 1997. Seasonal variation of amino acids and organic acids in the xylem sap of *Quercus ilex* L. growing on serpentine and sandy loam soils. In: Ando, T. et al. (Eds.), Plant Nutrition for Sustainable Food Production and Environment. Kluwer Academic, Japan, pp. 405–406.

Pohlmeier, A., 1999. Metal speciation, chelation and complexing ligands in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: From Molecules to Ecosystems. Springer, Berlin, pp. 29–50.

Prasad, M.N.V., 1998. Metal–biomolecule complexes in plants: occurrence, functions and applications. Analusis 26, 25–28.

Prasad, M.N.V., 1999. Metallothioneins and metal binding complexes in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: From Molecules to Ecosystems. Springer, Berlin, pp. 51–72.

Prasad, M.N.V., Freitas, H., 1999. Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils. Electronic Journal of Biotechnology 2, 36–50. Online Journal at website: http://ejb.ucv.cl or http://ejb.org.

Prasad, M.N.V., Hagemeyer, J., 1999. Heavy Metal Stress in Plants — From Molecules to Ecosystems. Springer, Berlin.

Prakasham, R.S., Merrie, J.S., Sheela, R., Saswathi, N., Ramakrishna, S.V., 1999. Biosorption of chromium VI by free and immobilized *Rhizopus arrhizus*. Environmental Pollution 104, 421–427.

Randall, J.M., 1977. Variations in effectiveness of bark as scavengers for heavy metal ions. Forest Production Journal 27, 51–56.

Randall, J.M., Garret, V., Bermann, R.L., Waiss, A.C., 1974. Use of bark to remove heavy metal ions from waste solutions. Forest Production Journal 24, 80–84.

Randall, J.M., Reuter, F.W., Waiss, A.C., 1975. Removal of cupric ions from solution by contact with peanut skins. Journal of Applied Polymer Science 19, 1563–1571.

Randall, J.M., Hautala, E., Waiss, A.C., Tschernitz, J.L., 1976. Modified barks as scavengers for heavy metal ions. Forest Production Journal 26, 46–50.

Roberts, E.J., Rowland, S.P., 1973. Removal of mercury from aqueous solutions by nitrogen containing chemically modified cotton. Environmental Science and Technology 7, 552–555.

Ross, S.M., 1994. Toxic Metals in Soil Plant Systems. Wiley, Chichester, UK.

Roy, D., Greenlaw, P.N., Shane, B.S., 1993. Adsorption of heavy metals by green algae and ground rice hulls. Journal of Environmental Science and Health Part A 28, 37–50.

Sequeira, E.M.D., Pinto da Silva, A.R., 1991. The ecology of serpentinized areas of northeast Portugal. In: Roberts, B.A., Proctor, J. (Eds.), The Ecology of Areas with Serpentinized Rocks. A World Review. Kluwer Academic Publishers, Dordrecht, pp. 169–197.

Shukla, S.R., Sakhardande, V.D., 1991. Metal ion removal by dyed cellulosic materials. Journal of Applied Polymer Science 42, 829–832.

Townsley, C.C., Ross, I.S., Atkins, A.S., 1986. Copper removal from a simulated leach effluent using filamentous fungus *Trichoderma viride*. In: Eccles, H., Hunt, S. (Eds.), Immobilization of Ions by Biosorption. Ellis Horwood, Chichester, pp. 159–170.

Vázquez, G., Antorrena, G., González, J., Doval, M.D., 1994. Adsorption of heavy metals ions by chemically modified *Pinus pinaster* bark. Bioresource Technology 48, 251–255.

Yetis, U., Ozcengiz, G., Dilek, F.B., Ergen, N., Erbay, N., Dolek, A., 1998. Heavy metal biosorption by white-rot fungi. Water Science and Technology 38, 323–330.